INVESTIGATIONS ON INSECTICIDES AS FEED ADDITIVES FOR THE CONTROL OF THE HOUSE FLY, *Musca domestica*

BY

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INTRODUCTION AND REVIEW OF LITERATURE

The house fly, Musca domestica Linn. is a well known pest and vector of diseases of man and animals. Many studies have been conducted on its biology and control, but relatively few, using the approach of incorporating insecticides as feed additives to kill fly larvae which develop in the manure. This study was stimulated further by the spread of the face fly, Musca autumnalis DeGeer, into Kansas. However, since face flies were not available at the time of this study house flies were used in all experiments. Treece (1961), in Ohio, found adult face flies to be more susceptible to insecticides than adult house flies.

The face fly differs from other cattle pests such as the horn fly and the stable fly in that it does not bite and suck blood. Instead it has sponging mouth parts similar to the house fly. Face flies cluster around the nose and eyes and cause irritation to the animals by feeding on the mucous secretions. It is thought that the face fly is involved in the transmission of pink eye in cattle, but this has not been proved. Since this fly clusters on the animals' heads it is difficult to control. The face fly has breeding habits similar to the horn fly in that it deposits its eggs in fresh cow droppings.

The feed-additive idea is not new. Gallagher (1928) attempted fly control by adding various substances in the drinking water of cattle and his attempts were unsuccessful. Knipling (1938) achieved control of horn flies by the addition of phenothiazine at 100 mg./kg. of body weight to the rations of cattle. Bruce (1939) continued work with phenothiazine and established the minimum lethal dose as 22 mg./kg. of body weight to prevent larval

development of horn flies in the feces. Bruce (1940) screened many compounds as possible feed additives for control of horn flies. From this screening, the most effective compound was rotenone fed at 0.3 grams per 100 pounds of body weight. Bruce (1942) in another series of tests found that zinc oxide would inhibit larval development of horn flies if fed daily to cattle at 1.5 grams per 100 pounds of body weight. After 1942, interest in feed additives for control of fecal breeding insects decreased. Then with the production of new insecticides such as the chlorinated hydrocarbons, Eddy (1954) found that lindane at 100 ppm, dieldrin at 25 ppm, and aldrin at 25 ppm would inhibit the development of the house fly, horn fly, and stable fly in the feces. Dunn (1959) and Harvey (1960) added Bacillus thuringiensis Berliner to the rations of cattle and achieved some control. Tracy (1960) determined that 50 micrograms of DDVP per gram of feces were required to inhibit larval growth of the house fly. Anthony et al. (1961) and Eddy (1961) found that CO-RAL and Bayer 22408 were the best of the materials tried and were effective at dosages as low as 1 mg./kg. The dosages of DDVP for this experiment were extrapolated from studies by Durham et al. (1957) and Tracy et al. (1960).

This study was designed to investigate (1) the effectiveness of insecticide additives in cattle rations to inhibit the development of house fly larvae in the droppings; and (2) certain toxicological aspects on the host of long term feeding of insecticides.

MATERIAL AND METHODS

Design

This study was divided into two parts: CO-RAL and DDVP treatments. The CO-RAL portion was carried out in conjunction with a larger experiment to study the effects of including CO-RAL (O,O-diethyl-O-3-chloro-4-methyl-2-oxo-2H-1benzopyran-7-yl phosphorothioate) in the fattening ration of feeder cattle for fly control (Skaptason and Pitts, 1962). Bioassays were performed to determine the toxicity of the droppings to house fly larvae.

The DDVP portion consisted of administering oral doses of 25 percent DDVP XP-472 resin formulation (O,O-dimethyl O-2,2-dichlorovinyl phosphate) to cattle, measuring blood cholinesterase activity, and bioassaying the feces with house fly larvae to determine the efficacy in controlling fecal breeding insects. The DDVP Study is further subdivided into experiments 1, 2 and 3.

Experimental Animals

CO-RAL Study. One hundred and twenty steers were divided into 12 pens of ten head each. Three replicates (pens) were used at each of the three treatment levels, and three pens for controls. These animals were housed in a feed lot in Kansas City, Kansas, and maintained on medicated feed during the experiment, from June 8, 1961 to September 4, 1961. Feeding was done under the direction of J. S. Skaptason.

DDVP Study. Five non-lactating dairy animals were purchased at a local auction, consisting of four Holsteins of 1000 pounds each, and one Jersey weighing approximately 700 pounds. The animals were held three weeks before

the experiment was started to ascertain that they were in good health and acclimated to the surroundings. During this period it was determined by a veterinarian that animals 2, 3, and 5 were six months pregnant. After the animals had been on treatment for two weeks the remaining non-pregnant animals were bred to an Angus bull. The animals were housed at Kansas State University Veterinary Research Laboratory in a dry lot. There was adequate shelter with a concrete floor and the pen was cleaned whenever necessary. Each animal received 5 pounds of complete dairy ration daily and all of the prairie hay desired. The dairy ration consisted of 500 parts corn, 400 parts bran, 300 parts oats, 400 parts soya, 300 parts alfalfa meal, 50 parts calcium, 10 parts las-o-min, 20 parts salt and 200 parts molasses, all based on dry weight. Las-o-min is a vitamin-mineral premix. There was a constant supply of water and salt.

Administration of Compounds

CO-RAl Study. CO-RAL was incorporated into a protein supplement at three levels designed to produce concentrations of 5, 10, 50 ppm, in the final ration. This ration was fed daily throughout the experiment.

DDVP Study. A twenty-five percent DDVP XP-472 formulation was administered orally by 1 ounce gelatin capsules with a balling gun. The dosages were weighed-out daily. Treatment was started on June 27, 1961 and continued through August 28, 1961. The four treatment animals were started at 1.5 grams of 25 percent DDVP XP-472 daily. Table 5 indicates the actual insecticide received daily and the total for the experiment. This treatment, designated DDVP Experiment 1, was continued until July 19, 1961. At this time the dosages were increased by staggered increments and

are designated DDVP Experiment 2. The actual insecticide received is indicated in Table 7. The animals remained at this level until July 26, 1961 at which time each treatment was increased 0.5 gram; it was then designated DDVP Experiment 3. The actual insecticide received is indicated in Table 9. They remained at this level through August 28, 1961 at which time the experiment was concluded. The animals were treated at approximately 8:30 a.m. throughout the experiment.

Collection of Samples

CO-RAL Study. Five fecal samples were selected at random following each of the three treatments: 5, 10, and 50 ppm., along with samples from the controls, and sent to Kansas State University for bioassay evaluation. The date and number of samples taken are indicated in Table 3. These samples were removed by gloved hand, manually extracted.

DDVP Study. The date and number of blood samples taken are indicated in Plates I, II, and III. Five ml. blood samples were taken by venipuncture from the external jugular vein and placed in a tube containing 0.1 ml. of heparin. The samples were then taken to the laboratory and refrigerated until the cholinesterase levels were determined, usually within 24 hours.

The fecal samples were taken as previously indicated and placed in marked pint food cartons and taken to the laboratory for bioassay. The date and number of fecal samples taken are indicated in Tables 4, 6, and 8.

Bioassay

Larvae were reared by a modified CSMA procedure and adult flies were fed on powdered non-fat dry milk and sugar at 1:1 ratio and supplied

separately with water. Eggs were collected from the adult flies the day before the fecal samples were taken. The eggs were placed on moist filter paper in a covered petri dish and held at 80 F. until the following day for hatching.

The frozen samples (CO-RAL) were allowed to thaw at room temperature and the fresh samples (DDVP) were used immediately. Each fecal sample was thoroughly mixed and a 100 gram portion placed in an 8 ounce Dixie cup. Twenty-five newly hatched larvae were counted with a camel hair brush and placed on a small piece of moist filter paper which was inverted on the fecal sample. The cup was placed in a wide mouth quart fruit jar with a screen top and maintained at a constant temperature of 80 F.

If any of the fecal samples were of high water content, dry CSMA medium was added to give more optimum consistency for larval growth.

Two days after implantation of larvae, the filter paper was removed and a layer of saw dust placed over each sample for a pupation site. The samples were then held for three weeks and at that time counts were made on the number of adult flies that had emerged.

Cholinesterase Determinations

Cholinesterase activity of the blood samples was determined by the colorimetric method reported by Robbins et al. (1953). Briefly the procedures were as follows. A standard curve was obtained for the substrate, acetylcholine bromide, by plotting optical densities against μ moles present. Then 0.05 ml. of heparnized whole bovine blood was pipetted into 0.95 ml. of saponin solution and allowed to react with 1 ml. of .004 M acetycholine bromide for 10 minutes. The reaction was stopped with alkaline hydroxylamine

and color was developed with hydrochloric acid and ferric chloride and read on a spectrophotometer at 540 mm. A non-specific color blank and a non-enzymatic reaction was run at the same time. The number of μ moles of acetylcholine bromide hydrolyzed was calculated by subtracting the μ moles of substrate remaining in the experimental samples from the total μ moles initially present as represented by the non-enzymatic control.

RESULTS

CO-RAL Study

Data are presented in Table 3. An analysis of variance is given in Table 1, which indicates that all F tests were significant.

Table 1. Analysis of variance of CO-RAL fly emergence data.

Source	d. f.	M. S.	F	Sig.
Treatments	3	436.46	70.40	***
Dates	3	43.58	7.03	***
T D	9	16.68	2.69	*
Error	48	6.20		

Table 2 shows where the differences lie as indicated by the analysis of variance. All treatment levels of CO-RAL gave significantly reduced fly emergence compared to the controls. The 50 ppm. level reduced emergence significantly more than the 5 ppm. (85 percent vs. 98 percent). There was no significant difference in emergence between the 10 ppm. (89 percent) and the 50 ppm. (98 percent), nor was the 5 ppm. significantly different from the

10 ppm.

Table 2. Table of means of dates and treatments of CO-RAL Study.

Treatment	Dates 6/24/61	7/24/61	8/12/61	8/21/61	Av.
0	12.5	5.5	12.2	15.5	11.60
5	1.0	0.5	3.2	3.2	2.00
10	2.0	0.2	0.0	3.8	1.50
50	0.0	0.2	0.5	0.8	0.19

Treatment LSD 1.772

Table 3. Emergence of house flies from feces from cattle fed CO-RAL. 1961.

Date Larvae Planted[a]	Rep. No.	Emergence of adult flies at dosages (ppm.): 0	5	10	50	Percent control (ppm.)[b]: 5	10	50
June 24	1	15	0	3	0			
	2	13	4	4	0			
	3	10	0	0	0			
	4	12	0	1	0			
	x	12.5	1.0	2.0	0	92	84	100
July 24	1	10	1	1	0			
	2	3	0	0	0			
	3	3	0	0	1			
	4	6	1	0	0			
	x	5.5	0.5	0.2	0.2	91	96	96
Aug. 12	1	11	3	0	0			
	2	10	1	0	0			
	3	15	5	0	1			
	4	15	4	0	0			
	x	12.8	3.2	0	0.5	75	100	96
Aug. 21	1	13	6	1	0			
	2	21	3	14	0			
	3	14	1	0	0			
	4	14	3	0	0			
	x	16.5	3.2	3.8	0	81	77	100
$\bar{x}$ all dates		11.8	1.9	1.5	0.2	85	89	98

[a] 25 larvae per sample

[b] percent control based on actual emergence in 0 ppm.

DDVP Study

Experiment 1. Table 4 indicates that, when all animals received 1.5 grams of 25 percent DDVP, bioassays resulted in 77 percent control compared to the controls.

Table 4. Emergence of house flies from feces from cattle fed 25 percent DDVP XP-472 at 1.5 grams daily. 1961.

Date Larvae Planted[a]	Emergence of adult flies Replicate No.					Percent control[b]
	Control	1	2	3	4	
July 3	7	3	1	0	1	
July 4	5	1	1	0	1	
July 5	4	2	1	0	1	
July 10	5	2	1	1	0	
July 11	4	3	0	1	0	
July 12	6	2	1	1	1	
July 17	4	1	0	1	1	
July 18	5	2	1	1	0	
July 19	4	3	0	1	3	
Average, all dates	4.9	2.1	0.7	0.7	0.9	77

[a] 25 larvae per sample

[b] percent control based on actual emergence from the controls.

Cholinesterase Levels. The blood cholinesterase activity of the DDVP treated animals are graphed in Plate I. Levels are plotted against days and are expressed in μmoles of acetylcholine bromide hydrolyzed. The average ChE depression at the end of DDVP Experiment 1 was 40 percent.

EXPLANATION OF PLATE I

Blood cholinesterase levels of four cows receiving 1.5 grams of 25 percent DLVP XP-472 formulation per day for twenty-two days. The control animal received none of the formulation.

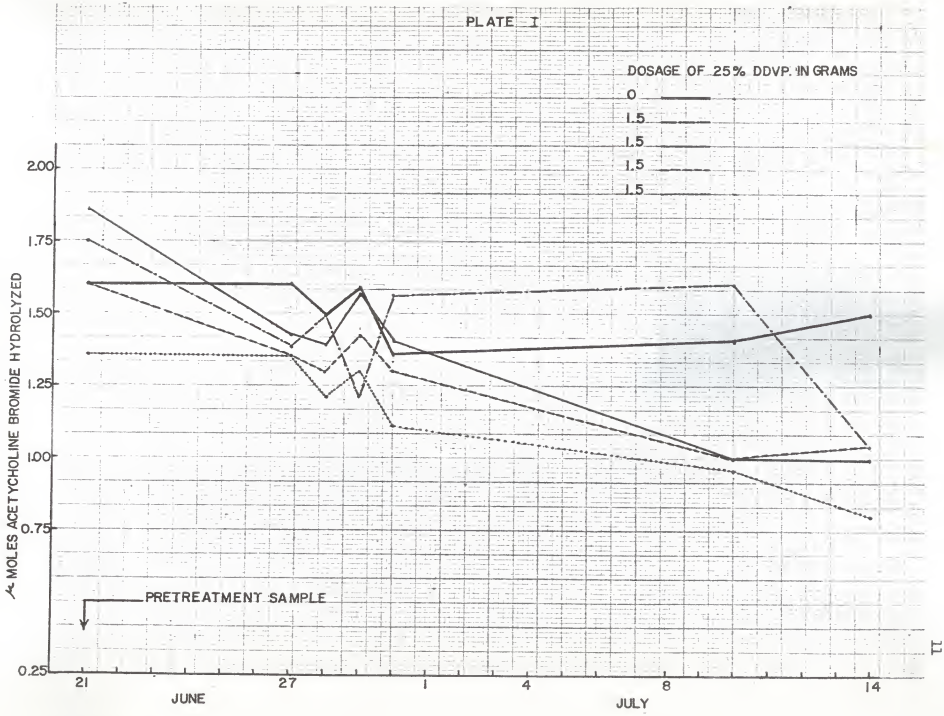
PLATE I
DOSAGE OF 25% DDVP. IN GRAMS
0
1.5
1.5
1.5
1.5
2.00
1.75
1.50
1.25
1.00
0.75
0.25
MOLES ACETYCHOLINE BROMIDE HYDROLYZED
PRETREATMENT SAMPLE
21
27
1
4
8
14
JUNE
JULY

Table 5 indicates the daily dosage of insecticide and the total insecticide received.

Table 5. DDVP Experiment 1, insecticide received from June 27 through July 18, 1961.

Animal No.	Daily dosage 25% DDVP in g.	Technical DDVP per day in mg.	Total technical DDVP for 22 days in g.
1	0	0	0
2	1.5	375	8.25
3	1.5	375	8.25
4	1.5	375	8.25
5	1.5	375	8.25

Experiment 2. Bioassay. Table 6 indicates that, when the dosages were staggered (1.5 to 3.0) there appeared to be enhanced control with increase in dosage. Based on percent control, the greatest difference was between 1.5 grams daily and 2.0 grams daily of 25 percent DDVP.

Table 6. Emergence of house flies from feces from cattle fed 25 percent DDVP XP-472 daily. 1961.

Date Larvae Planted[a]	Emergence of adult flies at dosages (g.)					Percent control[b]			
	0	1.5	2.0	2.5	3.0	1.5	2.0	2.5	3.0
July 22	5	3	0	0	1				
July 24	0	0	0	0	0				
July 25	2	0	0	0	0				
Average, all dates	2.3	1.0	0	0	0.3	57	100	100	87

[a] 25 larvae per sample

[b] percent control based on actual emergence of the controls.

Cholinesterase Levels. Levels for DDVP are graphed in Plate II. Levels are plotted against days and expressed in μmoles of acetylcholine bromide hydrolyzed. The average ChE depression at the end of DDVP Experiment 2 was 46 percent. Table 7 indicates the daily dosage of insecticide and the total insecticide received.

Table 7. DDVP Experiment 2. Insecticide received from July 19 through July 25, 1961.

Animal No.	Daily dosage 25% DDVP in g.	Technical DDVP per day in mg.	Total technical DDVP for 7 days
1	0	0	0
2	1.5	375	2.62
3	2.0	500	3.50
4	2.5	625	4.37
5	3.0	750	5.25

Experiment 3. Bioassay. Table 8 indicates emergence data. In this experiment the results were very erratic. The lowest dosage gave 25 percent control while the highest dosage gave 55 percent control, and one of the intermediate dosages gave 63 percent control.

EXPLANATION OF PLATE II

Blood cholinesterase levels of four cows receiving 1.5, 2.0, 2.5 or 3.0 grams of 25 percent DIVP XP-472 formulation daily for 7 days. The control animal received none of the formulation.

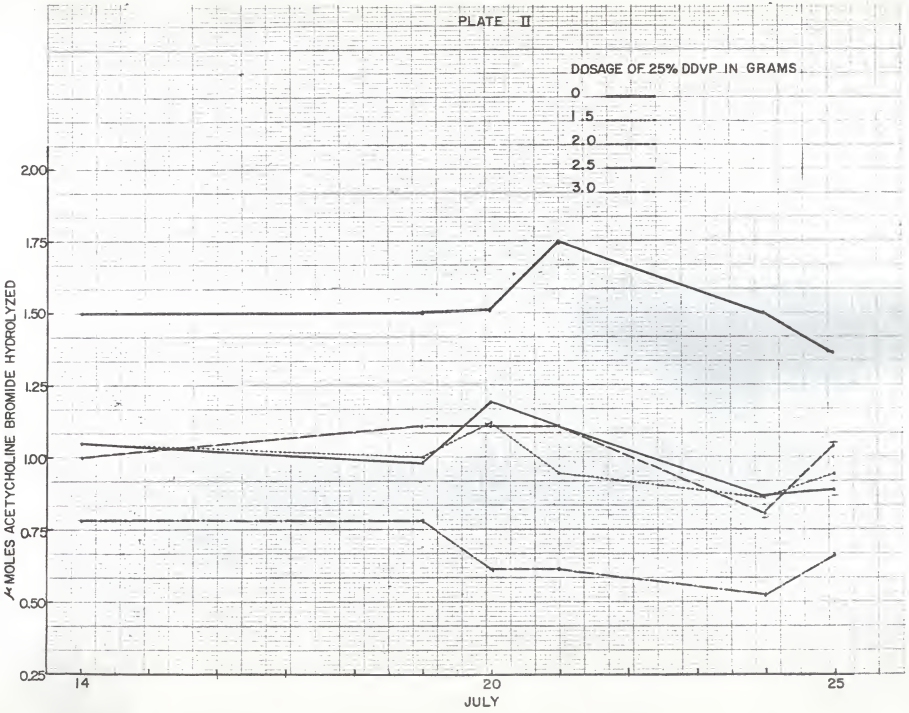
PLATE II
DOSAGE OF 25% DDVP IN GRAMS.
0
1.5
2.0
2.5
3.0
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
μ MOLES ACETYCHOLINE BROMIDE HYDROLYZED
14
20
25
JULY

Table 8. Emergence of house flies from feces from cattle fed 25 percent DDVP XP-472 daily. 1961.

Date Larvae Planted[a]	Emergence of adult flies at dosages (g.)					Percent control[b]			
	0	2.0	2.5	3.0	3.5	2.0	2.5	3.0	3.5
July 26	10	8	12	3	4				
Aug. 11	5	5	5	3	3				
Aug. 12	9	5	7	3	4				
Average, all dates	8.0	6.0	8.0	3.0	3.6	25	0	63	55

[a] 25 larvae per sample

[b] percent control based on actual emergence of controls

Cholinesterase Levels. The levels for DDVP Experiment 3 are in Plate 3. Levels are plotted against days and expressed in μmoles of acetylcholine bromide hydrolyzed. The average ChE depression at the end of DDVP Experiment 3 was 55 percent. Table 9 indicates the daily dosage of insecticide per animal and the total insecticide received.

Table 9. DDVP Experiment 3. Insecticide received from July 26 through August 28, 1961.

Animal No.	Daily dosage 25% DDVP in g.	Technical DDVP per day in mg.	Total technical DDVP for 34 days in g.
1	0	0	0
2	2.0	500	17.00
3	2.5	625	21.25
4	3.0	750	25.50
5	3.5	875	29.75

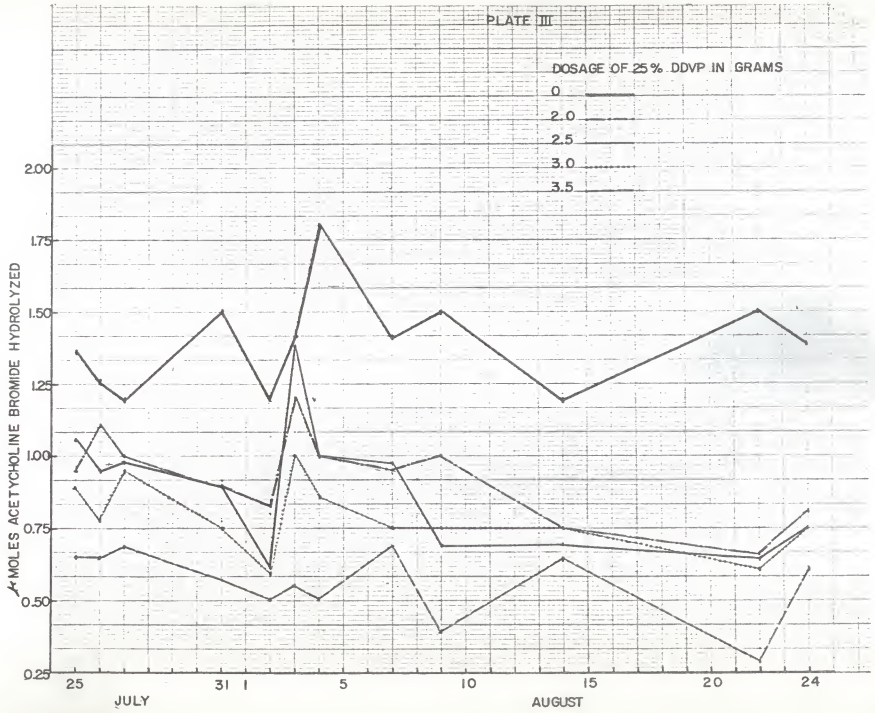
PLATE III
DOSAGE OF 25% DDVP IN GRAMS
0
2.0
2.5
3.0
3.5
MOLES ACETYCHOLINE BROMIDE HYDROLYZED
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
25
31
1
5
10
15
20
24
JULY
AUGUST

Table 10 indicates the total insecticide fed during DDVP Experiments 1, 2, and 3, which ranged from totals of technical DDVP of 27.87 and 43.25, over a 63 day feeding period.

Table 10. Total 25 percent DDVP XP-472 fed during a 63 day feeding period.

Animal No.	Grams of 25% DDVP-XP-472 fed during 63 days	Grams of technical DDVP fed during 63 days
1	0	0
2	111.5	27.87
3	132.0	32.90
4	152.5	38.12
5	173.0	43.25

DISCUSSION

Bioassay

The CO-RAL bioassays indicate that CO-RAL has possibilities as a long term feed additive for the control of house flies. None of the dosages tried, gave complete control but the degree of control was slightly enhanced with the increase in dosage and fly breeding would be expected to be substantially reduced if an area control program were performed.

In all DDVP bioassays, emergence in the controls were so low that it is impossible to make any conclusions on the efficacy of the DDVP formulations. This poor emergence in the controls may be explained by the diet the animals received. The diet of the DDVP animals was predominately hay whereas the CO-RAL animals were receiving a high grain diet with protein supplement. This difference in diet may have had an effect upon the rate at which moisture

was lost from the fecal samples. If true, it would explain the difference in emergence in the controls. Other than diet, the fecal samples were treated the same and in some cases evaluated at the same time.

Cholinesterase Levels - DDVP Study

In the DDVP Experiment 1, all animals received 1.5 grams of 25 percent DDVP XP-472 for twenty-two consecutive days. During this period the blood cholinesterase levels were gradually reduced to 40 percent of their pre-treatment values. Subsequently after the dosages were staggered (Experiment 2), there was only slight depression, the greatest depression at the highest dosage. In DDVP Experiment 3, all dosages were increased by 0.5 grams. This increase depressed all levels to some extent but with no serious reductions.

It appears that after low level feeding of insecticides under these experimental conditions, blood cholinesterase levels gradually drop to a certain point and then level off. This may be accounted for in that the formation or reactivation cholinesterase has reached a steady state with the amount of inhibitor available to the enzyme. No toxic symptoms from DDVP were observed.

On September 1, 1961, the animal that had received the highest dosage of insecticide had a calf. A blood sample was taken shortly after birth and cholinesterase level was determined and found to be 0.60 μmoles. This value corresponds to the level of the mother which was 55 percent below pretreatment value. The calf was examined and no apparent abnormalities found. Animal number 3 also had a calf and was normal in all respects.

Animal number 4 was sacrificed and autopsied for gross pathology. The examination was negative. The fetus, which was approximately 3 months along,

was examined and appeared to be normal.

From the autopsy report and the fact that 2 normal calves were born to the animals from the highest level of treatment, it appears that long term feeding of 25 percent DDVP XP-472 has no adverse effect on unborn young or any of the internal organs.

SUMMARY

The efficacy of CO-RAL (O,O-diethyl-O,3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) and DDVP (O,O-dimethyl O-2,2-dichlorovinyl phosphate) in controlling house flies in droppings of cattle receiving daily doses of the insecticides was investigated. Certain toxicological aspects of long term feeding of DDVP were also investigated.

Bioassays of the feces from cattle receiving CO-RAL in the rations produced significant control at 5, 10, and 50 ppm. The degree of control was only slightly enhanced by increased dosage. It appears that CO-RAL has promise as a feed additive for an adjunctive control measure for insects breeding in fresh cow droppings in an area control campaign.

Bioassays of the feces of animals receiving different levels of 25 percent DDVP XP-472 indicated some control but were inconclusive primarily because of low emergence in the control samples. This was probably due to the diet of the animals and differences in moisture content of the feces.

Cholinesterase levels of the DDVP treated animals exhibited gradual depression with low dosages but there was no serious reductions. After the levels had reached 40 percent to 55 percent of normal, a steady state was maintained.

Two calves were born to DDVP treated animals and both appeared normal.

An autopsy was performed on one animal at the end of the experiment, and the gross pathology was negative.

Results indicate no ill effects produced by feeding of DDVP for periods up to 63 days at the dosages used, but additional work will have to be done to determine the efficacy of DDVP in controlling house flies in the droppings.

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requirements for the degree

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1962

During the summer of 1961, studies were initiated to investigate the efficacy of CO-RAL (O,O-diethyl-O-3- chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) and DDVP (O,O-dimethyl-2,2-dichlorovinyl phosphate) for control of house flies in the feces of cattle receiving daily dosages of the insecticides. Certain toxicological aspects of long-term feeding of DDVP were also investigated.

Studies involved bioassays of fresh feces and determination of blood cholinesterase levels. Evaluations consisted of implantion of 25 newly hatched house fly larvae into a 100 gram sample of feces and adult fly emergence counts after three weeks. Degree of control was determined by comparing the emergence of treated samples with the untreated. Cholinesterase levels were determined by a colorimetric method.

CO-RAL was fed to 120 steers at 0, 5, 10, and 50 ppm. in the total ration from June 8 to September 4, 1961. Bioassays of the feces indicated significant control at all treatment levels. Degree of control was only slightly enhanced by increased dosage. CO-RAL has promise as a feed additive for an adjunctive control measure for flies breeding in fresh cow droppings in an area control program.

DDVP was administered to five dairy cows at rates from 0 to 3.5 grams of 25 percent DDVP XP-472 daily from June 27 through August 28, 1961. Bioassays indicated some control but were inconclusive primarily because of low emergence in the control samples. This was probably due to the diet of the animals and differences in moisture content of the feces between the CO-RAL and DDVP studies.

Cholinesterase levels of all DDVP treated animals exhibited gradual depression, but there were no serious reductions. After the levels had

reached 40 percent to 55 percent of normal, a steady state was maintained.

Two calves were born from DDVP-treated mothers soon after the experiment was concluded; both appeared normal. A blood sample taken from one calf soon after birth showed a cholinesterase level of 0.60 μ moles, which coincided with that of the mother. An autopsy was performed on one cow that received 38.12 g. of tech DDVP during 63 days. The gross pathology was negative.

No ill effects were produced by feeding DDVP for period up to 63 days, but more work will have to be done to determine the efficacy of DDVP in controlling house flies in the droppings.